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Effect of Drinking Water Chlorination on Campylobacter spp. Colonization of Broilers

N. J. Stern,[A] M. C. Robach,[B] N. A. Cox,[A] and M. T. Musgrove[B]

[A]USDA—Agricultural Research Service, Richard B. Russell Agricultural Research Center, Poultry Microbiological Safety Research Unit, 950 College Station Road, Athens, GA 30604-5677
[B]Wayne Farms LLC, 4110 Continental Drive, Oakwood, GA 30566

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SUMMARY. The main source for *Campylobacter* spp. transmission from the environment to broiler chickens is still unclear. One implicated reservoir for the organism has been untreated broiler drinking water. This study was conducted with broilers first using experimental conditions (isolation units) and second under commercial conditions. We compared the rate of intestinal colonization in chickens provided 2 to 5 parts per million (ppm) chlorinated drinking water in relation to the frequency of colonization in chickens given unsupplemented drinking water. No significant difference ($P > 0.05$) was detected in isolation frequency or level of *Campylobacter* spp. colonization in birds provided chlorinated drinking water and control birds provided water without supplemental chlorine. In the isolation unit experiments, 86.3% (69/80) of the control and 85.0% (68/80) of the treated birds were colonized at levels corresponding to an average of $10^{5.2}$ and $10^{5.1}$ log colony-forming units (cfu) *Campylobacter* spp./g of cecal contents, respectively. Additionally, two sets of paired 20,000 bird broiler houses, with and without chlorination (2–5 ppm chlorine), were monitored in a commercial field trial. Effectiveness of chlorination was judged by prevalence of *Campylobacter* spp. in fecal droppings (960 samples) taken from the flocks in treated and control houses. Birds from the control houses were 35.5% (175/493) *Campylobacter* spp. positive, while 45.8% (214/467) of the samples from the houses having chlorinated drinking water yielded the organism. Chlorination of flock drinking water at the levels tested in this study was not effective in decreasing colonization by *Campylobacter* spp. under commercial production practices presently used in the United States.

RESUMEN. Efecto de la adición de cloro al agua de bebida sobre la colonización por Campylobacter en pollos de engorde.

La fuente de transmisión del *Campylobacter* desde el medio ambiente a los pollos de engorde no se ha determinado todavía. Se sospecha que uno de los reservorios para el microorganismo es el agua de bebida no tratada. El presente estudio se realizó primero en condiciones de laboratorio (unidades de aislamiento) y luego en condiciones de campo. Se comparó la frecuencia de la colonización intestinal en pollos que recibieron agua tratada con cloro con niveles de 2 a 5 partes por millón (ppm), con la de pollos que recibieron agua sin tratar. No se encontró una diferencia significativa ($P > 0.05$) en la frecuencia de aislamientos y los niveles de colonización por el *Campylobacter* entre los dos grupos. En las unidades de aislamiento, el 86.3% de las aves (69/80) en el grupo control, y el 85.0% de las aves (68/80) en el grupo tratado con el agua clorinada presentaron niveles de colonización promedios de $10^{5.2}$ y $10^{5.1}$ unidades formadoras de colonia del *Campylobacter* spp por gramo de contenido cecal, respectivamente. Adicionalmente, se estudió el efecto de la administración de agua de bebida con y sin tratamiento con cloro (a niveles de 2 a 5 ppm) en galpones de crianza comerciales con capacidad para 20 000 aves. La efectividad del tratamiento se determinó mediante la evaluación de los niveles de prevalencia del *Campylobacter* spp en las 960 muestras de heces tomadas de los galpones de las aves tratadas y los grupos control. Se aisló el *Campylobacter* en el 35.5% (175/493) de las muestras tomadas de los galpones de aves del grupo control, mientras que los niveles de aislamiento en las aves tratadas fue de un 45.8% (214/467). Estos resultados indican que los niveles de tratamiento con cloro usados en este estudio no son efectivos para la disminución de los niveles de colonización por parte del *Campylo-*

bacter spp en las parvadas criadas bajo las prácticas de producción actuales en los Estados Unidos.

Key words: broilers, *Campylobacter* spp., chlorination, water

Abbreviations: cfu = colony-forming units; IU = isolation unit; ppm = parts per million

Campylobacter spp., commonly isolated from commercial broiler flocks worldwide, is associated with handling of raw poultry and consumption of undercooked poultry (7,11). Water contaminated with *Campylobacter* spp. has been suggested as an environmental factor that may be important in the colonization of broiler flocks with the organism (8). After discounting vertical transmission, litter, feed, small animals, and flock-to-flock cross-contamination, Pearson *et al.* (8) concluded that water was the principal source for the spread of the organism on a commercial farm. The organism was detected by filtration and fluorescent antibody techniques, but was never cultured. However, naive chicks raised in isolation and given water from the farm became colonized by the same serotype of *Campylobacter* spp. as was isolated from the farms. Furthermore, the researchers purported that by sanitizing water storage tanks with quaternary ammonium and chlorinating water, the prevalence of *Campylobacter* spp. in subsequent flocks dropped from 81 to 7%. Within weeks of terminating drinking water chlorination, the prevalence of *Campylobacter* spp. colonization on the farm returned to >80%.

The purpose of this study was to determine whether broiler drinking water supplemented with chlorine at levels known to kill *Campylobacter* spp. would be effective in controlling broiler colonization. This treatment proved ineffective in experiments conducted in controlled isolation units and in commercial field trials.

MATERIALS AND METHODS

Isolation unit trials. *Chickens.* Day-of-hatch broiler chicks were obtained from a local commercial hatchery. The birds were placed into isolation units (IUs) with raised wire floors and ventilated with positive pressure filtered air. Ten chicks were placed in each IU and provided feed and water *ad libitum.* Wire floors allowed fecal and cecal droppings to drop out of the chick's reach to prevent challenge of the *Campylobacter* spp. by ingestion of feces. This experiment consisted of four separate trials, each including two challenge levels (described below) for both control and treated chicks. A total of 160 chicks was used.

Treatment. Water was provided to the chicks by nipple waterers that were connected to clean glass containers holding either untreated water or water supplemented with 2 parts per million (ppm) chlorine (Clorox™ Bleach; Clorox Co., Oakland, CA). Water was changed daily. Level of the chlorination was monitored using a "DPD chlorine outfit" (LaMotte Chemical, model LP-31R, Chestertown, MD) chlorination test kit.

Challenge Organism. A 24-hr subculture of *Campylobacter jejuni* strain 969 (isolated from a chicken) was used to prepare challenge cultures for the chicks. Cultural suspensions were prepared from isolates grown on Brucella-FBP agar, incubated microaerobically (5% O_2, 10% CO_2, 85% N_2) at 42 C, and diluted in buffered peptone water. Birds were challenged with either 10^3 or 10^5 cells by oral gavage.

Microbiological Sampling. Approximately 5 days after challenge, chicks were killed by cervical dislocation and ceca were aseptically removed. Each cecal sample was diluted (1:3) with buffered peptone water and mixed before serial dilutions were plated onto Campy-Cefex agar plates (10). Following incubation for 36–48 h at 42 C in microaerobic atmosphere (5% O_2, 10% CO_2, 85% N_2), plates were observed for presumptive *Campylobacter* spp. colonies. Representative colonies were confirmed by microscopic observation of wet mount preparations for typical cellular morphology and motility. Confirmed colonies were counted and converted to $\log_{10}$.

Field trials. *Commercial Farms.* Two farms were selected primarily because they contained two parallel commercial broiler houses. The houses were typical of those found in north Georgia, having dirt floors covered by pine litter shavings, open screened windows, doors that remained open for ventilation, automatic feeders, and nipple drinkers (without cups). Litter from the previous flock was changed before houses were restocked. Chicks placed on pairs of farms were from the same parent flocks. The facilities had limited biosecurity measures (no foot baths, hand washing, or protective foot wear in use). Broiler drinking water was provided by drilled wells (>250 ft deep). The drinking water system had an in-line medicator that was used to provide a constant 2 to 5 ppm chlorine in the drinking water of the "treated" houses. To ensure that the medicator was in proper

Table 1. Prevalence and levels of *Campylobacter* spp. in cecal contents of week-old chicks (10/treatment group) challenged with either 10^3 or 10^5 *Campylobacter* spp. and provided treated (2–5 ppm of chlorine) or control (no supplementation) drinking water in isolation units.

		Control		Treated	
Challenge level[A]	Trial	% +	log cfu/g ceca	% +	log cfu/g ceca
10^3	1	90	5.66	30	1.92
	2	100	5.63	90	4.00
	3	40	2.39	90	5.96
	4	70	3.91	100	5.83
Average		75	4.40	78	4.38
10^5	1	90	6.06	70	4.91
	2	100	6.03	100	6.33
	3	100	6.03	100	6.33
	4	100	6.03	100	5.83
Average		98	6.04	93	5.83

[A]Approximate number of *Campylobacter* cells used to orally challenge each chick.

Table 2. Weekly prevalence of *Campylobacter* spp. in the feces of commercial broilers from two pairs of farms where the birds were supplied water containing 5 ppm chlorine (treated) or unsupplemented water (control) during broiler production.

		Control		Treated	
	Week	+/*n*	% +	+/*n*	% +
Farm A	1	0/50	0	0/0	0
	3	1/43	2	12/42	37
	4	12/46	26	39/45	87
	5	23/46	50	35/47	74
	7	23/39	59	18/30	60
	8	19/23	83	23/24	96
Sum		104/247	42	127/238	53
Farm B	1	0/34	0	0/26	0
	2	0/42	0	0/41	0
	3	4/50	8	11/46	24
	4	1/30	3	0/26	0
	5	14/30	47	12/30	40
	6	25/30	83	26/30	87
	7	27/30	90	38/30	93
Sum		71/246	29	87/229	38

working order, chlorine levels were monitored every 2 wk.

Sampling. Clean disposable gloves were used to collect 20 to 50 randomly distributed fecal droppings at approximately weekly intervals from placement to slaughter. Samples were placed in sterile screw-capped test tubes and transported on ice to the laboratory. Buffered peptone water was used to dilute the samples (1:3), and dilutions were spread plated on Campy-Cefex agar and incubated as described previously (10).

Statistical analysis. The data derived from the treatments were tested using the general linear model procedure of SAS. Significance was defined as $P < 0.05$. Prevalence data were analyzed by the chi-square test for independence.

RESULTS

Results from the isolation unit experiments appear in Table 1. There was no significant statistical difference in prevalence or colonization levels between treated and control groups whether chicks were challenged with 10^3 or 10^5 cells of *Campylobacter* spp.

For the commercial field trials, prevalence of broiler colonization was determined by sampling of feces (Table 2). Rates of colonization for control and treated birds were not significantly different for either trial as determined by chi-square analysis.

DISCUSSION

Environmental contamination has been regarded as the principal source of infection for newly placed broilers and turkeys (9). Water has been implicated as an important environmental source in the colonization of broilers with *Campylobacter* spp. (4,8). Though the organism is highly sensitive to chlorination (2,13), not all farms use chlorinated water. Even when water is chlorinated, organic matter can bind chlorine in solution, making it unavailable for a bactericidal effect (6). In a preliminary isolation unit experiment (data not shown), chlorination was ineffective when birds defecated in the dish of bell drinkers used to provide chlorinated water. In the isolation unit trials reported in the current experiment, in order to maximize the killing potential of chlorine, birds were provided chlorinated water via nipple drinkers. This modification did not enhance the effectiveness of the treatment. However, challenge levels used in the isolation experiment were considerably higher than birds would be exposed to in naturally contaminated water (5).

Commercial field trials were conducted to determine the effectiveness of chlorination in suppressing or eliminating colonization in broiler flocks. On both farms, chlorination of drinking water for the treated group was the

only husbandry practice altered between the paired houses. Houses using nipple drinkers were selected. However, there was no delay in the onset or prevalence of colonization in the treated flocks as compared to the control flocks. These results would support that contaminated water was not the most important factor in colonization of the commercial broiler flocks surveyed.

Drinking water may be involved in flock colonization by *Campylobacter* spp. Kapperud *et al.* (4) sampled 176 broiler flocks at slaughter. They then conducted retrospective interviews on broiler husbandry practices and determined that not disinfecting water was associated with an increased risk of colonization. The prolonged survival of the organism in water has been demonstrated in the form of a viable nonculturable state and in biofilms that can form on surfaces (3,12), including pipes and storage tanks. However, if the drinking waters in the current experiment were truly the most important source of flock infection, we would have anticipated differences in colonization between the chlorine treated and untreated flocks. Pearson *et al.* (8) strongly suggest the dominant role of contaminated water in their farm study. However, many significant differences exist in husbandry practices between birds described by Pearson and those under consideration in our work. On the farms studied by Pearson *et al.,* litter was mechanically removed between flocks and houses were washed with a high-pressure washer, disinfected, and then fumigated prior to subsequent placement of chicks. Broiler flock husbandry in the United States does not include these practices. Though Pearson *et al.* provided data supporting the contribution of drinking water to broiler colonization, perhaps the husbandry practices used in the United States create other environmental reservoirs that play a more significant role in colonization of flocks by *Campylobacter* spp. After performing epidemiological surveys of Swedish broiler flocks, Berndtson *et al.* (1) concluded that water was not a factor in broiler colonization under husbandry practices observed in that country. In the present study, chlorination of drinking water was not shown to be effective as the sole means to control broiler colonization by *Campylobacter* spp.

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